Bull. Environ. Contam. Toxicol. (2005) 74:1118–1125

DOI: 10.1007/s00128-005-0697-9

Bulletin of Environmental Contamination and Toxicology

Histopathological Changes in the Hepatopancreas of *Palaemonetes turcorum* (Holthuis, 1961) (Crustacae, Decapoda), Exposed to Lead Acetate

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Received: 15 June 2004/Accepted: 9 March 2005

Lead is a potent environmental toxicant frequently found in air, drinking water, soil, dusts, lead-based paints and industrial by products. Exposure to lead from any of these sources by ingestion, inhalation or dermal contact can cause significant toxicity (Singh 1993; Chadzynski 1986).

The biological impact of heavy metals in aquatic systems has become a major concern in recent years. The important food sources of invertebrates or fish, accumulate heavy metals through the food chain. Effects of lead and other heavy metals have been studied in many invertebrates (Kutlu and Sümer 1998; Kutlu et al. 2002; Kutlu et al. 2003; Meyer et al. 1991; Méndez et al. 1997).

The liver and pancreas in vertebrate animals are the main sites of both organic and inorganic reserve material. In crustaceans, the hepatopancreas has the function of both the liver and pancreas of vertebrates and therefore is involved in the secretion of digestive enzymes and the absorption and storage of lipid material. Hepatopancreas could also be a potential indicator organ for heavy metal toxicity (Papathanassiou and King 1984). The crustacean hepatopancreas is composed of blind tubules, which are internally lined with a single epithelial layer delimiting the lumen and consisting of at least four cell types: E (embryonic), R (resorptive), F (fibrillar) and B (blister-like) cells. All the other cells are derived from E cells and have different functions in metabolic processes (Zilli et al. 2003).

The common prawn *Palaemonetes spp.* is an ecologically important and widely distributed shallow water species which has a potential economic importance. In the present study, we chose *Palaemonetes turcorum*, an endemic species in Yeşilhan region in Turkey, as a sensitive organism to environmental pollutants. The examination of ultrastructural changes in the hepatopancreas of *P. turcorum* due to acute lead toxicity is important to determine the effects of this heavy metal on cellular structure and organelles clearly, in a short period of time.

This study suggests that the effects of acute lead toxicity can be seen clearly as some histopathological changes in the cells of hepatopancreatic ceca of digestive system in *P. turcorum*, since hepatopancreas could be a potential indicator organ for heavy metal toxicity.

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Effects of heavy metal lead were investigated by using both light and electron microscopy techniques. The results obtained are discussed in view of estimating the direct consequences of acute lead toxicity in hepatopancreatic cells of *P. turcorum* on ultrastructural level.

MATERIALS AND METHODS

Specimens of *Palaemonetes turcorum* (Holthuis 1961) were collected from a branch of the Sakarya River basin in Yeşilhan region, located in 36 th km. of Eskişehir-Ankara road in Turkey. Animals were acclimatized under laboratory conditions for 7 days prior to use. They were maintained in an aquarium with aerated tap water with pH 7.5-8.2, DO 5.6 ± 0.2 mg/L, total hardness 260 mg/L (as $CaCO_3$), alkalinity 45.084 mg/L and temperature 23.0 ± 0 ^{0}C under a static system. The acclimatized animals were transferred from the stock tank into glass jars with 1 liter each of water. The treatment conditions and water were the same as that for acclimatization.

The experiment consisted two groups; control group and experimental group. There were four replicates per group with five animals with an average body weight each of 1.21 ± 0.1 g in each replicate. Animals were starved for 96 hr.

Pb solutions were prepared by dissolving lead (II) acetate trihydrate ($Pb(CH_3COO)_2 \cdot 3H_2O$) (Fluka 153449) in distilled water. The animals in experimental group were exposed to lead at a single toxicant concentration LC_{50} (96 hr) 0.394 mg/L. This concentration of LC_{50} was determined for *Gammarus pulex*, another freshwater organism sensitive to environmental pollution from Crustacea, by Kutlu and Sümer (1998). The test water was not renewed during the experiment. Animals in control group were subjected to the same procedures, but exposed only to clean, conditioned tap water.

Light microscopic and ultrastructural analysis were applied for animals both in the control group and experimental group. The best pictures are shown in this paper.

For microscopic study, the hepatopancreas was dissected out and fixed in glutaraldehyde fixative (4.5 %) pH 7.2-7.3 for 24 hr. The samples were then taken into sodium phosphate buffer pH 7.4 for 15 min. They were treated with OsO_4 2 % for 2 hours and then washed in sodium phosphate buffer 2 times for 15 min. The samples were then dehydrated in a graded series of alcohols. In order to harden the tissues they were treated with propylene oxide 2 times for 30 min. The samples were then embedded in araldite / propylene oxide (1:1) for 1 hr and sectioned at 800 nm (0.8 microns) thickness and stained with toluidin blue. Semi-thin sections were observed using an Olympus light microscope. For electron microscopic study, ultrathin sections were cut by Leica Ultracut R ultramicrotom using glass knife and stained with uranylacetate 2% and lead citrate 4 %. They were examined under a Jeol 1220 transmission electron microscope (Bancroft and Gamble 2002).

RESULTS AND DISCUSSION

The hepatopancreas is typically an exocrine gland and its microscopic structure differs basically and completely from that of a true liver (Van Weel 1974). The crustacean hepatopancreas is composed of blind tubules, which are internally lined with a single epithelial layer delimiting the lumen and consisting of at least four cell types: E (embriyonic), R (resorptive), F (fibrillar) and B (blister-like) cells. E cells are undifferentiated, and show mitotic activity. All the other cell types are derived from E cells. R cells are the most abundant cell type and are in nutrient absorption. The F cells play role in the synthesis and secretion of digestive enzymes. B cells are thought to be the main site for the accumulation of waste products (Fig 1) (Zilli et al. 2003).

Effects of lead were observed in the hepatopancreatic cell types as some structural changes. There were loss of the cell outline, karyolysis, pyknosis and enlarged vacuoles (Fig 2). We observed some changes in organelles such as the dilation of granular endoplasmic reticulum (GER), increase in number of vacuoles, lysosomes, number and size in lipid droplets and myelin bodies (Fig 3). The similar lesions were observed in the animals in experimental group and 100 % of the shrimp had those cellular changes.

Lead also is an electrophile that avidly forms covalent bonds with the sulfhydryl group of cysteine in proteins. Thus, proteins in all tissues exposed to lead will have lead bound them. Some proteins became labile as lead binds with them because lead causes the tertiory structure of the protein to change; cells of the nervous system are particularly susceptible to this effect. Some lead-bound proteins change their tertiary configuration sufficiently so that they become antigenic; renal tubular cells are particularly susceptible to this effect because they are exposed to relatively high lead concentrations during clearance (Burtis and Ashwood 1994).

Depending on these changes in organelles, their special functions are surely affected. For instance mitochondrial damages affect ATP synthesis, the changes occured in endoplasmic reticulum (ER) influence protein synthesis and its transport (Kutlu et al. 2002). Increase in number of vacuoles, lysosomes, lipid droplets and myelin bodies are often considered as nonspesific stress responses. The accumulation of lipid droplets in the cytoplasm results from the decline of protein synthesis that accompanies cellular injury. When heavy metals enter a cell, they stimulate the cell to produce defensive stress induced lysosomes. Heavy metals can also destroy other organelles such as mitochondrion and lysosomes. Lysosomes digest these membranous structures in the cell through the action of autophagosomes resulting in the presence of myelin bodies which were found in more destroyed cells. Lead can kind with a negative charge present on the organel membrane leading to a change in cell membrane permeability.

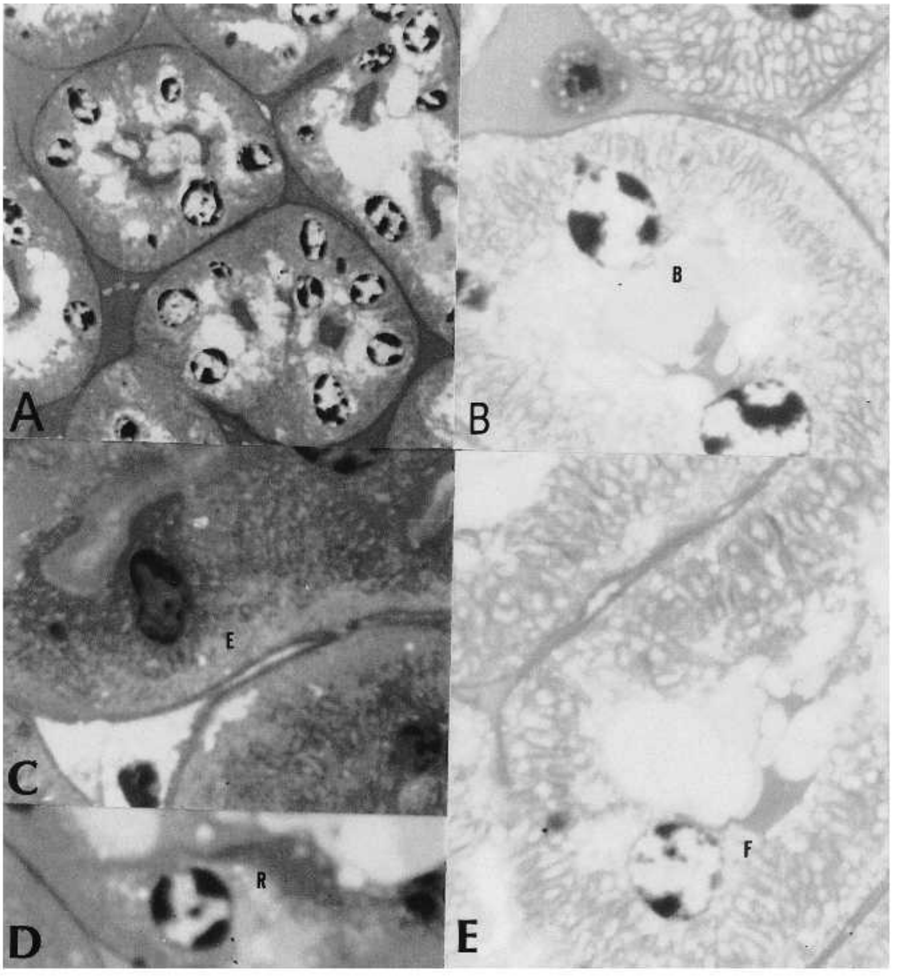

Figure 1. Light micrographs of the hepatopancreatic tubule in control group A) Hepatopancreatic tubule cross–section (X 40 Toluidin blue). B). High magnification of secretory cell (B cell). C) High magnification of embryonic cell (Ecell). D) High magnification of resorptive cell (R cell). E) High magnification of fibrillar cell (F cell).

We observed an increase in the number of lysosomes, lipid droplets, myelin bodies and vacuolation in the cells of *P. turcorum* hepatopancreas. There are many studies of ultrastructural alterations induced by heavy metals in aquatic environments (Kutlu et al. 2002; Kruatrachue et al. 2003; Zilli et al. 2003).

In their study similar to ours, Meyer et al. (1991) studied the biochemical and histochemical observations on effects of low-level metal load (lead and cadmium) in different organ systems of the freshwater crayfish *Astacus astacus* L. (Crustacea:Decapoda). They found heavy metal deposits in the apical cytoplasm

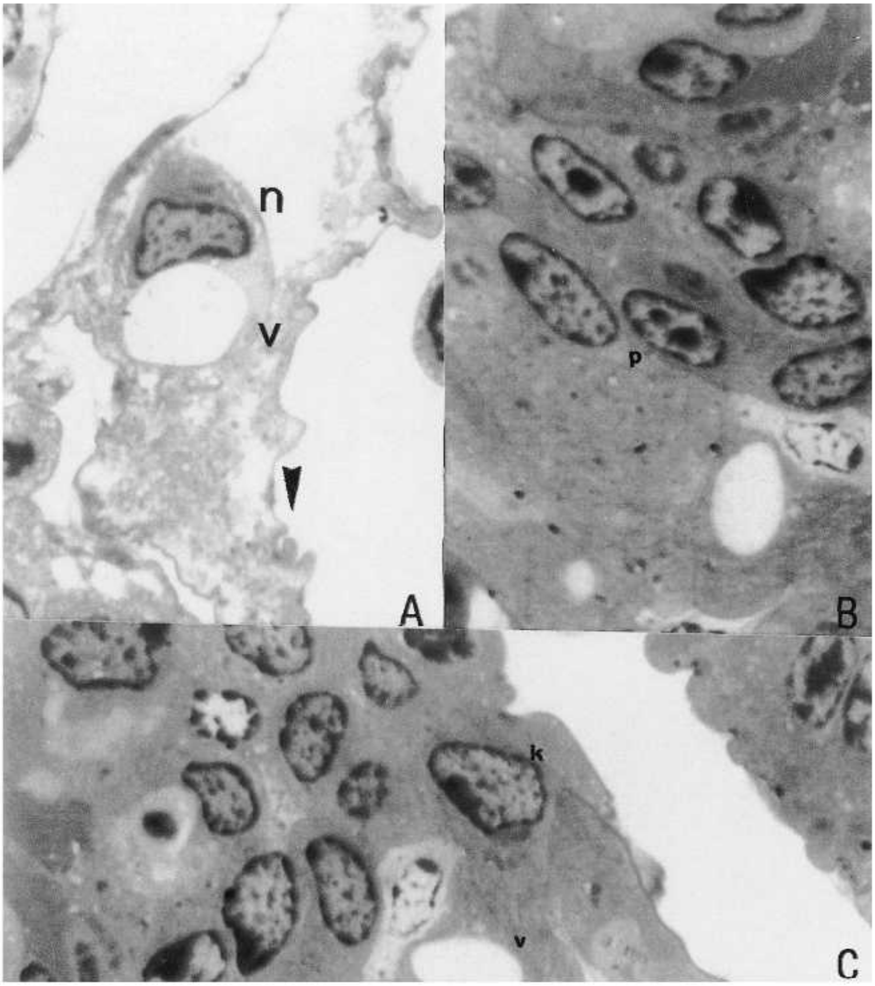

Figure 2. Light micrographs of the hepatopancreatic cell in experimental group. A) High magnification of deformed B cell with enlarged vacuole (v) and karyolysis in nucleus (n), loss of the cell outline (▼). B) Pyknosis (p) in F cell. C) Karyolysis (k) in E cell and vacuolation (v) in cytoplasm (X 100 Toluidin blue).

of the epithelial cells of digestive gland and also large vacuoles were seen in parallel with our results. Yang and Chen (2003) studied the hepatocyte ultrastructure of common carp after gallium exposure and observed lipid inclusions, nucleus deformation, increase in the number of secondary lysosomes and ultrastructural alterations of the organelles. Besides the studies with invertebrates, previous reports demonstrated some changes due to the effects of heavy metals. Krautrachue et al. (2003) determined vacuolation, loss of cell outline, increase in the number of lipid droplets, lysosomes and myelin bodies in the gastrointestinal tract of fish, *Puntius goniotus* fed on dietary cadmium. Effects

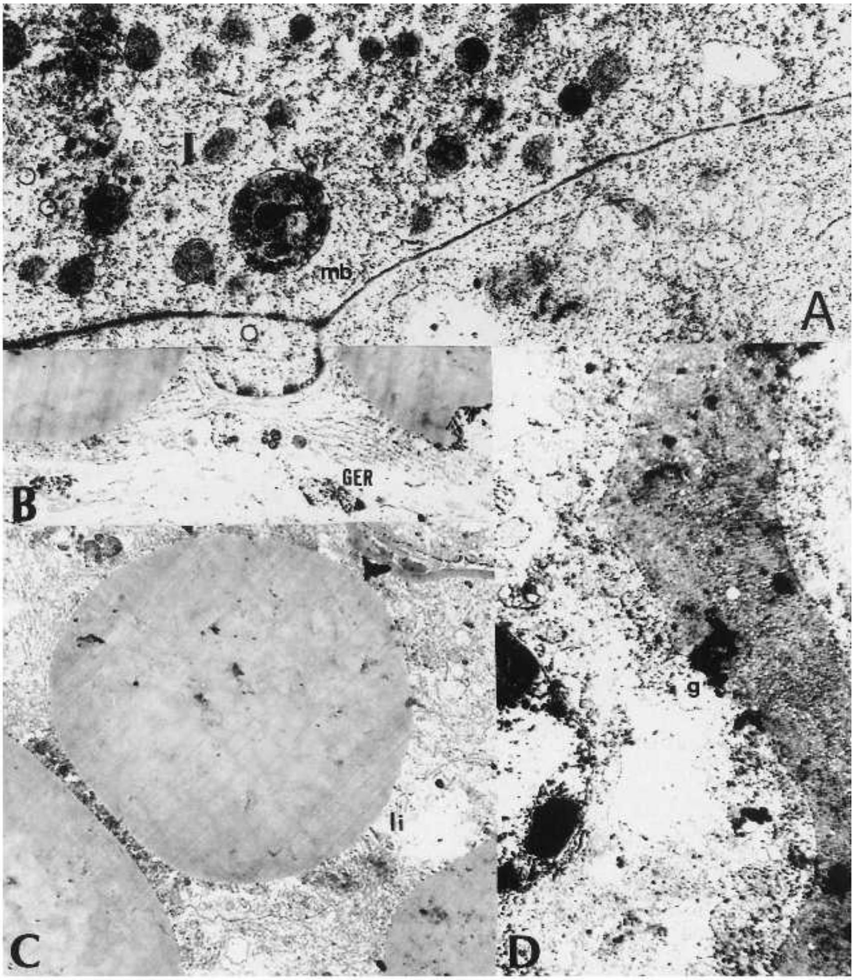

Figure 3. Transmission electron micrographs of hepatopancreatic cell of *Palaemonetes turcorum*. A) Lysosome (l) and myelin body (mb) (X 2500). B) Dilation of granular endoplasmic reticulum GER (X 2500). C) Increase in number and size in lipid droplets (li) (X 2000). D) Glycogen particles (g) (X 4000).

of starvation were investigated on the fine structure of the hepatopancreas in the common prawn *Palaemon serratus* (Pennant) by Papathanassiou and King (1984). The results of that study occurred as the changes in the structure and number of the organelles in the different cell types of the hepatopancreas. However, effects of starvation on heavy metal toxicity should be investigated in the further studies for invertebrates.

In this study, effects of acute lead toxicity were observed clearly in the hepatopancreas of *P. turcorum* such as the nuclear changes, dilation of membranous structures, increase in the number of vacuoles, lysosomes, number and size of lipid droplets, myelin bodies and glycogen particles. Lead exposure results in serious damage on hepatopancreas of *P. turcorum*. The metal may consequently inhibit the physiological functions of this organ. The major findings of this study brings up the importance of the hepatopancreas of *P. turcorum* as an accumulator of lead and an indicator organ for acute lead toxicity.

Invertebrates are generally more sensitive to pollutants than either fish or algae. The shrimp, *P. turcorum* is not consumed by humans but is a major prey organism of and is used as a bait for commercially important edible fish. Concentrations of heavy metals in this shrimp could be amplified up through the food chain to man. In further studies, it can be useful to perform more detailed histopathological studies and enzymatic studies in the hepatopancreas of *P. turcorum* exposed to lead toxicity in order to support the findings of this study.

Acknowledgments. This work was supported by Anadolu University Research Fund. We are thankful to Mrs. Arzu İşcan for her help in preparing tissues for the electron microscopy.

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